Transmission Geometry for Atmospheric Pressure Matrix-Assisted Laser Desorption Ionization

John H. Callahan[1], Akos Vertes[2], and Marsha C. Galicia[2]

[1]Naval Research Laboratory, Code 6115, Washington DC 20375
[2]The George Washington University, Chemistry Department, Washington DC 20052

In both atmospheric pressure matrix-assisted laser desorption ionization (AP-MALDI) and vacuum MALDI, the laser typically illuminates the analyte on the front side of an opaque surface (reflection geometry). Another configuration consisting of laser illumination through the sample back side (transmission geometry) has been used in conventional MALDI; however, its use and the number of reports in literature is limited.[1] The use of transmission geometry with AP-MALDI is demonstrated here. Such a geometry is simple to implement, eliminates the restriction for a metallic sample holder and allows for the potential analysis of samples on their native transparent surfaces, e.g., cells or tissue sections on slides.

METHODS

A home built transmission geometry AP-MALDI source was interfaced to a Finnigan LCQ ion trap mass spectrometer as seen in Figure 1. Both conducting and insulating transparent slides served as sample substrates. The slide was sandwiched between two metal plates in order to apply a high voltage. The metal plates had center holes to allow laser illumination to the sample. The sample was positioned directly (~3 mm) in front of the ion trap orifice. A nitrogen laser beam was focused through a lens to illuminate the sample from behind causing sample desorption and ionization. Instrumental and sample preparation parameters (e.g., applied voltage, matrix type, deposition method) were varied throughout the experiment.

RESULTS AND DISCUSSION

Spectra obtained from transmission and reflection geometry for AP-MALDI are compared in Figure 2. Differences can be seen in the mass spectra. First, the transmission configuration yields a lower intensity signal (about an order of magnitude lower). In reflection mode, analyte-matrix clusters are present; however, these peaks are undetectable when using transmission geometry. Using Angiotensin I (ATI) as the test compound, several parameters, e.g., laser power, substrate surface/material, applied voltage, and sample preparation, were investigated to ascertain optimal conditions. Two different laser intensities were used. The laser used in reflection geometry gives about 25 μJ/pulse. This was insufficient to detect analyte in the transmission mode. Instead, a higher laser power was needed (150 μJ/pulse to 190 μJ/pulse). Three types of substrate surfaces/materials, i.e., conductive (quartz), nonconductive (quartz and glass), and silanized (glass), were examined. No significant differences in the ion detection were seen between the various surfaces/materials. The substrate conductivity, surface, and composition did not effect the spectra obtained by transmission geometry. Different MALDI matrices were also tested. The MALDI matrices α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), and sinapinic acid (SA) were premixed with the ATI analyte. The CHCA produced better and more reproducible spectra than the other matrices. The effect of sample voltage was determined by applying voltages between 0 kV to 2.5 kV. Ions were not detected without a voltage applied to the sample. This serves as an indication of voltage assisted ion transport toward the orifice of the mass spectrometer. With voltages of 1.5 kV to 2.5 kV, high ion signals could be achieved. In order to assess the effect of crystallite size and layer thickness on ion production, dried droplet and electrospray sample deposition methods were tried. While the dried droplet method typically produces larger crystallites in non-uniform distribution, electrosprayed samples are composed of homogeneously distributed smaller/thinner crystallites. However, both deposition methods produced similar ion yields and similar quality spectra. Further optimization is necessary to detect analytes other than ATI. We have demonstrated that AP-MALDI generates detectable ion signal in transmission geometry. Success is evident through the detection of ATI by AP-MALDI on an ion trap mass spectrometry in transmission configuration. Transmission geometry AP-MALDI may have a great impact in the direct analysis of samples pre-deposited on transparent surfaces, e.g., biological materials on microscope slides.

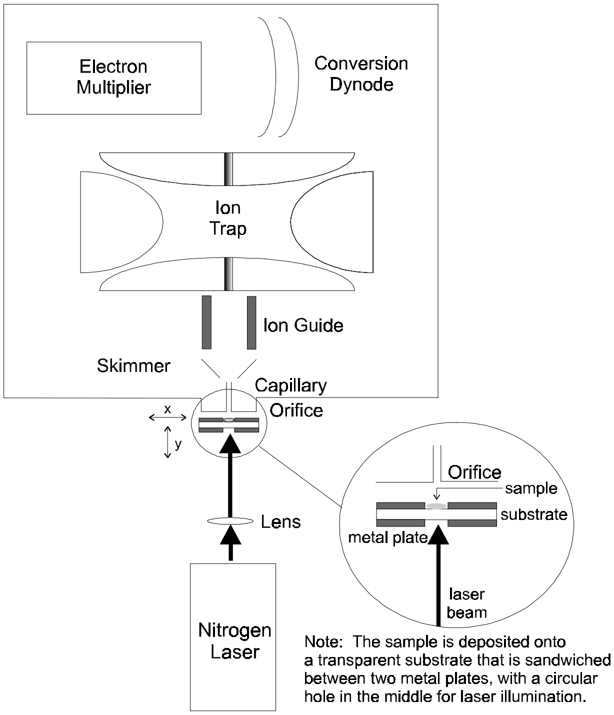

FIGURE 1. Instrumental setup for AP-MALDI transmission geometry experiments.

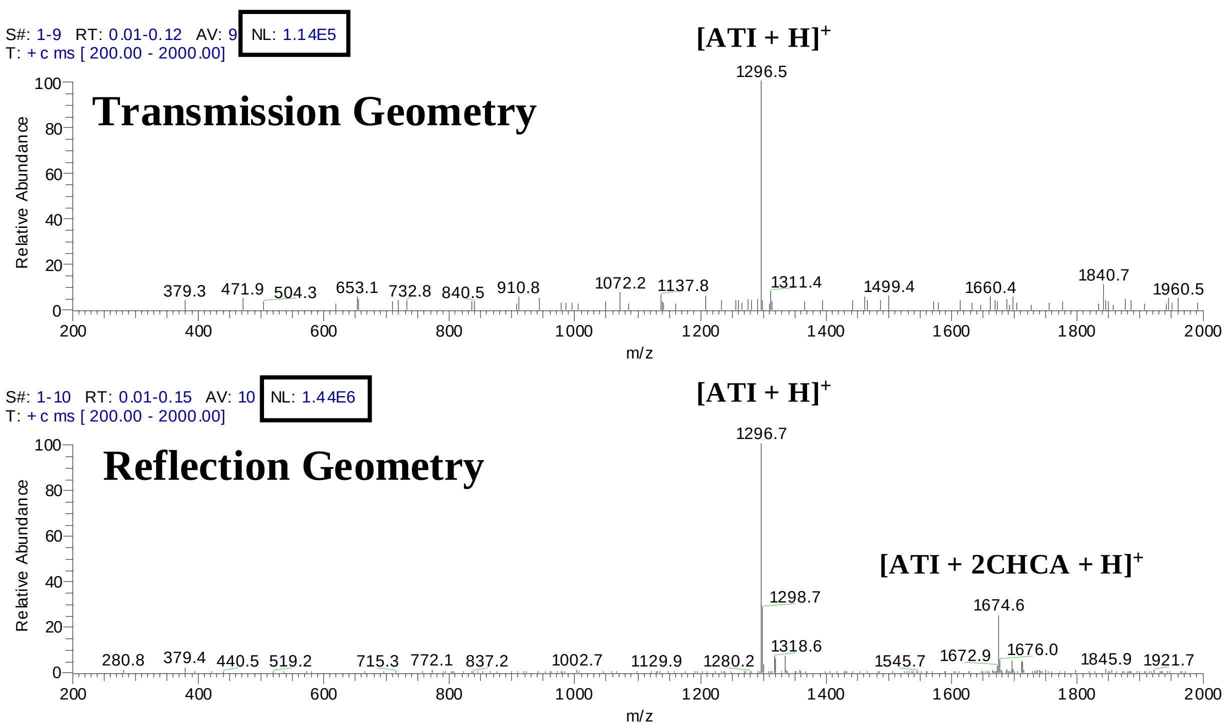

FIGURE 2. Comparison of ATI spectra obtained from transmission geometry (top) and reflection geometry (bottom) AP-MALDI IT MS.

---

[1]A. Vertes, L. Balazs and R. Gijbels, *Rapid Comm. Mass Spectrom.*, 1990, **4**, 263-266.